

AD-A102 416

ARMY INST OF DENTAL RESEARCH WASHINGTON DC  
THE ANTIBACTERIAL EFFECTS OF CALCIUM HYDROXIDE APEXIFICATION PA--ETC(U)  
JUN 81 P M DIFIORE, D D PETERS

F/G 6/15

UNCLASSIFIED

NL

100-1  
100-100



END  
DATE  
FILMED  
8-81  
DTIC

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER <b>6</b>	2. GOVT ACCESSION NO. DA OH 6038	3. RECIPIENT'S CATALOG NUMBER <b>AD-A102416</b>
4. TITLE (and Subtitle) The Antibacterial Effects of Calcium Hydroxide Apexification Pastes on <i>Streptococcus sanguis</i> .		5. TYPE OF REPORT & PERIOD COVERED Submission of Paper Jan 81 - June 81
7. AUTHOR(s) Peter M. Difiore, Donald D. Peters, Jean A. Setterstrom, and Lewis Lorton		6. PERFORMING ORG. REPORT NUMBER
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Army Institute of Dental Research Walter Reed Army Medical Center Washington, DC 20012		8. CONTRACT OR GRANT NUMBER(s)
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research & Development Command HQDA-IS Fort Detrick, MD 21701		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS <b>16</b> 62775A/3S762775A825/00/006
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) <b>9 Kept. for</b> <b>1 Jan - June 81</b> <b>LEVEL</b>		12. REPORT DATE June 1981
16. DISTRIBUTION STATEMENT (of this Report) This document has been approved for public release and sale; its distribution is unlimited.		13. NUMBER OF PAGES 15
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		15. SECURITY CLASS. (of this report) UNCLASSIFIED <b>12 16</b>
18. SUPPLEMENTARY NOTES		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
19. KEY WORDS (Continue on reverse side if necessary and identify by block number)  Apexification pastes, <i>Streptococcus sanguis</i> .		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Four calcium hydroxide based apexification pastes were tested for their anti-bacterial effects on <i>Streptococcus sanguis</i> . Their zones of growth inhibition on blood agar plates were measured at 2, 4, 6, and 8 days. Only the camphorated parachlorophenol and the metacresylacetate pastes showed zones of inhibition. Both of these zones of inhibition decreased with time, however, the zones of inhibition for the parachlorophenol paste decreased at a slower rate.		

AD A102416

DTIC FILE COPY

DTIC  
ELECTRIC  
AUG 4 1981  
C

DD FORM 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

Accession For	
NTIS GRA&I	<input checked="checked" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By _____	
Distribution/	
Availability Codes	
Avail and/or	
Dist	Special
<b>A</b>	

The Antibacterial Effects of  
Calcium Hydroxide Apexification Pastes on  
*Streptococcus sanguis*

Peter M. DiFiore, D.D.S., M.S., MAJ, DC, USA\*  
Donald D. Peters, D.D.S., M.S., COL, DC, USA\*\*  
Jean A. Setterstrom, Ph.D.\*\*\*  
Lewis Lorton, D.D.S., M.S.D., LTC, DC, USA\*\*\*\*

UNITED STATES ARMY INSTITUTE OF DENTAL RESEARCH  
WALTER REED ARMY MEDICAL CENTER  
WASHINGTON, DC 20012

\*Endodontic Resident  
\*\*Director, Endodontic Residency Program  
\*\*\*Research Microbiologist  
\*\*\*\*Research Dental Officer and Assistant Research Coordinator

81 8 03 004

# ABSTRACT

Four calcium hydroxide based apexification pastes were tested for their antibacterial effects on *Streptococcus sanguis*. Their zones of growth inhibition on blood agar plates were measured at 2, 4, 6, and 8 days. Only the camphorated parachlorophenol and the meta-cresylacetate pastes showed zones of inhibition. Both of these zones of inhibition decreased with time, however, the zones of inhibition for the parachlorophenol paste decreased at a slower rate.

## INTRODUCTION

Calcium hydroxide, used as an apexification paste, was introduced in the United States by Kaiser<sup>1</sup> and Frank.<sup>2</sup> They used calcium hydroxide mixed into a paste with camphorated parachlorophenol.<sup>1,2</sup> Subsequently, much has been written about the use of this formula to induce closure of open apices.<sup>3,4</sup>

Other vehicles for calcium hydroxide have been used for apexification pastes. These are metacresylacetate,<sup>5</sup> methylcellulose,<sup>6</sup> normal saline,<sup>7,8</sup> and distilled water.<sup>9</sup>

Although camphorated parachlorophenol and metacresylacetate both have antibacterial properties, camphorated parachlorophenol is generally considered to be more antibacterial than metacresylacetate.<sup>10-12</sup>

Earlier bacteriological studies of infected endodontic systems showed a predominance of gram positive *Streptococcus viridans*,<sup>13</sup> while more recent studies have shown them to be anerobic infections of indigenous bacteria.<sup>14,15</sup> The cultivable bacteria from dental pyogenic infections have been shown to be a mixture of anerobic rods and cocci, and facultative streptococci.<sup>16</sup> Representative bacterial species from these groups have been grown on blood agar medium to test the antibacterial effects on various medicaments.<sup>17,18</sup>

At present, the antibacterial effects of the various calcium hydroxide pastes used for apexification have not been studied. Since the high pH of calcium hydroxide would make it antibacterial by itself, the question arises as to the necessity of using an antimicrobial or a neutral vehicle in the paste preparation.<sup>8,19</sup>

The purpose of this study was to investigate the antibacterial effects of four calcium hydroxide pastes made individually with camphorated parachlorophenol, metacresylacetate, methyl cellulose and water.

#### MATERIALS AND METHODS

The testing technique used for this investigation is based on the standardized single disk method for antibiotic susceptibility.<sup>20</sup> A culture of *Streptococcus sanguis* (gram positive, facultative anerobe) was reconstituted from lyophilization and grown aerobically in trypticase soy liquid medium. Five prereduced blood agar culture plates (15 x 150mm) were seeded with this culture. A combination of the pour plate and cotton swab streaking methods were used to insure a complete and even distribution of the bacterial culture. Five penny cylinders (hollow stainless steel tubes 10mm long and 8mm in diameter) were placed in an even distribution over the seeded blood agar plates (Fig I). The four calcium hydroxide pastes to be tested were individually mixed by spatulation on a glass slab using a sterile technique. Two grams of powdered calcium hydroxide USP and 1cc of the appropriate liquid (camphorated parachlorophenol, metacresylacetate, or sterile distilled water) was mixed to produce a thick paste consistent with that used clinically. Calcium hydroxide and methycellulose (Pulpdent) was used directly out of its syringe dispenser. A half gram of each paste was condensed into a penny cylinder in direct contact with the blood agar surface of each plate.

The open ends of the penny cylinders were sealed with Cavit. The fifth empty penny cylinder, with its open end sealed with Cavit, was used as a control. The five seeded blood agar plates with the test medicaments on them were incubated anerobically using disposable gas pac generators. The diameters of the zones of bacterial growth inhibition were measured with a millimeter caliper at 2, 4, 6, and 8 days.

#### RESULTS

There were no zones of growth inhibition for the calcium hydroxide and water paste, the calcium hydroxide and methycellulose, or for the control. There were zones of growth inhibition for the calcium hydroxide and camphorated parachlorophenol paste, and for the calcium hydroxide and metacresylacetate paste over all four time periods.

The measurement data, paired differences, averages, paired average differences, standard deviations and paired t-test results are shown in Table I.

The average zone diameters decreased for both the camphorated parachlorophenol and metacresylacetate pastes but the average zone diameter rate of decrease for camphorated parachlorophenol was less (Fig II).

The difference between their effects at each time interval was shown by t-test to be statistically significant (Table I).

The paired average differences of the diameters of the zones of

inhibition decreased at each time interval (table I)

The Pearson's  $r$  between time and decrease in zone of inhibition for the metacresylacetate paste was  $-0.6198$  and the camphorated para-chlorophenol paste was  $-0.3005$ . This difference is significant at the  $0.01$  level of significance.

The regression coefficients (metacresylacetate paste  $= -1.47$ , camphorated parachlorophenol paste  $= -0.644$ ) of the decrease in zone diameter over time between the two pastes was also statistically different ( $p < 0.01$ ).

#### DISCUSSION

The size of the zone of bacterial growth inhibition does not necessarily reflect the strength of the antibacterial agent. The zone size may be influenced by the molecular size of the chemical and its diffusion constant. An agent which diffuses more easily will give a larger zone. The fact that there was no inhibition zone for the water paste and Pulpdent may just mean that they did not adequately diffuse through the medium. Both camphorated para-chlorophenol and metacresylacetate have been shown to easily diffuse through blood agar medium.<sup>21</sup>

In comparing the results obtained with the camphorated para-chlorophenol paste and the metacresylacetate paste, two observations require some explanation. First, the likely explanation for the larger zones obtained with the metacresylacetate paste was that more free metacresylacetate was available for diffusion and not



that it was more antibacterial. Second, the slower rate of reduction in the size of the zones obtained with the camphorated parachlorophenol paste indicates that either it maintains its antibacterial effect longer or that camphorated parachlorophenol is being released more slowly and therefore it is available longer to cause its effect.

Since the periapical tissues of teeth with infected root canals are generally free of bacteria,<sup>22,23</sup> the important antibacterial effect is within the root canal system and not beyond the apical foramen. Diffusion may be an advantage in the micro-environment of the endodontic system where penetration through the dentinal tubules may expand antibacterial efficacy.<sup>24,25</sup>

In the clinical situation, the calcium hydroxide camphorated parachlorophenol paste sets in a cement-like manner,<sup>2</sup> while the calcium hydroxide metacresylacetate paste remains unset.<sup>5</sup> This may explain the greater availability of metacresylacetate to act initially while the camphorated parachlorophenol may be released more slowly and therefore may be available over a longer period of time. The slow release of camphorated parachlorophenol may allow for a minimal tissue irritational effect with a long term antibacterial effect. This may partially explain the excellent apical response reported with this mixture.<sup>2,4</sup>

*Streptococcus sanguis* was chosen as a general test organism for this study because it is an indigenous oral facultative anerobic microorganism that is conveniently obtained and easily

grown in the laboratory. This study evaluates the relative effects on the microorganism of calcium hydroxide mixed with various liquids. Camphorated parachlorophenol and metacresylacetate have been shown to cause consistent antimicrobial results irrespective of the type of test organisms utilized.<sup>11,18</sup> Therefore, for this study, it appeared that the use of the one microorganism would give the information desired.

#### SUMMARY AND CONCLUSIONS

Four apexification pastes (calcium hydroxide and camphorated parachlorophenol, calcium hydroxide and metacresylacetate, calcium hydroxide and methylcellulose (Pulpdent), and calcium hydroxide and distilled water) were tested for their antibacterial effects on *Streptococcus sanguis*, by measuring their zones of growth inhibition on blood agar plates over time periods.

1. Calcium hydroxide with distilled water and Pulpdent both showed no growth inhibition, whereas calcium hydroxide with camphorated parachlorophenol and calcium hydroxide with metacresylacetate both showed growth inhibition.

2. The inhibitory zones for both the camphorated parachlorophenol and metacresylacetate paste decreased with time, but the camphorated parachlorophenol paste decreased at a slower rate.

3. The camphorated parachlorophenol and metacresylacetate in calcium hydroxide pastes are capable of diffusion and thus expanding the area of antibacterial effect.

Reprint requests should be directed to:

Colonel Donald D. Peters  
Director, Endodontic Residency Program  
US Army Institute of Dental Research  
Walter Reed Army Medical Center  
Washington, DC 20012

MILITARY DISCLAIMER

Commercial materials and equipment are identified in this report to specify the investigative procedure. Such identification does not imply recommendation or endorsement, or that the materials and equipment are necessarily the best available for the purpose. Furthermore, the opinions expressed herein are those of the authors and are not to be construed as those of the Department of the Army or the Department of Defense.

## REFERENCES

1. Kaiser, H.J.: Management of Wide-Open Canals With Calcium Hydroxide. Presented at the American Association of Endodontics, Washington, DC, 1964.
2. Frank, Alfred L.: Therapy for the Divergent Pulpless Tooth by Continued Apical Formation. J Am Dent Assoc 72:87-93, 1966.
3. Steiner, J.C., and VanHassel, H.J.: Experimental Root Apexification in Primates. Oral Surg 31:409-415, 1971.
4. Torneck, Calvin D.; Smith, Jerry S.; and Grindall, Paul: Biological Effects of Endodontic Procedures on Developing Incisor Teeth. IV. Effects of Debridment Procedures and Calcium Hydroxide Camphorated Parachlorophenol in the Treatment of Experimentally Induced Pulp and Periapical Disease. Oral Surg 35:541-554, 1973.
5. Stewart, George G.: Calcium Hydroxide-Induced Root Healing. J Am Dent Assoc 90:793-800, 1975.
6. Heithersay, Geoffrey S.: Stimulation of Root Formation in Incompletely Developed Pulpless Teeth. Oral Surg 29:620-630, 1970.
7. Cvek, Mimir: Treatment of Non-Vital Permanent Incisor Teeth With Calcium Hydroxide. Odont Revy 23:27-44, 1972.
8. Cvek, Mimir; Hollander, Lars; and Nord, Carl-Erik: Treatment of Non-Vital Permanent Incisors With Calcium Hydroxide. VI. A Clinical, Microbiological, and Radiological Evaluation of

treatment in One Sitting of Teeth With Mature and Immature Root.  
Odont Revy 27:93-108, 1976.

9. Bennie, William H., and Rowe, A.H.R.: A Hist ological Study of the Periapical Tissue of Incompletely Formed Pulpless Teeth Filled With Calcium Hydroxide. J Dent Res 52:1100-1116, 1973.
10. Harrison, John W., and Madonia, John V.: Antimicrobial Effectiveness of Parachlorophenol. Oral Surg 30:267-274, 1970.
11. Van Der Wall, Gerald L.; Dowson, John; and Shipman, Charles, Jr.: Antibacterial Efficacy and Cytotoxicity of Three Endodontic Drugs. Oral Surg 33:230-241, 1972.
12. Sprangberg, L.; Engström, B.; and Langeland, Kaare: Biological Effects of Dental Materials. 3. Toxicity and Antimicrobial Effects of Endodontic Antiseptics In Vitro. Oral Surg 36:856-871, 1973.
13. Winkler, K.C., and vanAmerongen, J.: Bacteriologic Results From 4,000 Root Canals Cultures. Oral Surg 12:857-875, 1959.
14. Keudel, Kenneth; Conte, Marin; Fusimoto, Lynn; Earnfest, Michael; and Berry, Hugh G.: Microorganisms Isolated From Pulp Chambers. J Endod 2:146-148, 1976.
15. Sundquist, Goran: Umea University Odontological Dissertations No. 7., Bacteriological Studies of Necrotic Dental Pulps, 1976.
16. Sabiston, Charles B.; Gribbsby, William P.; and Segerstrom, Nancy: Bacterial Study of Pyogenic Infections of Dental Origin. Oral Surg 4:430-435, 1976.

17. Wesley, David Joseph; Marshall, F. James; and Rosen, Samuel:  
The Quantitation of Formocresol as a Root Canal Medicament.  
Oral Surg 29:603-612, 1970.
18. Treanor, Hugh F., and Goldman, Melvin: Bactericidal Efficiency  
of Intracanal Medications. Oral Surg 35:791-796, 1972.
19. Tronstad, L.; Andreasen, J.O.; Hasselgren, G.; Kristerson, L.;  
and Riis, I.: pH Changes in Dental Tissue After Root Canal  
Filling With Calcium Hydroxide. J Endod 7:17-21, 1981.
20. Bauer, A.W.; Kirby, W.M.M.; Sherris, J.C.; and Turek, M.:  
Antibiotic Susceptibility Testing by a Standardized Single  
Disk Method. Am J Clin Pathol 45:493-496, 1966.
21. Wantulok, John C., and Brown, Jack I.: An In Vitro Study of  
the Diffusibility of Camphorated Parachlorophenol and Metacresyl-  
acetate in the Root Canal. Oral Surg 34:653-660, 1973.
22. Melville, T.H., and Birch, R.H.: Root Canal and Periapical Floras  
of Infected Teeth. Oral Surg 23:93-98, 1967.
23. Block, Robert M.; Bushell, Adolph; Rodrigues, Homero; and  
Langeland, Kaare: A Histopathology Histobacteriologic and  
Radiographic Study of Periapical Endodontic Surgical Specimens.  
Oral Surg 42:656-678, 1976.

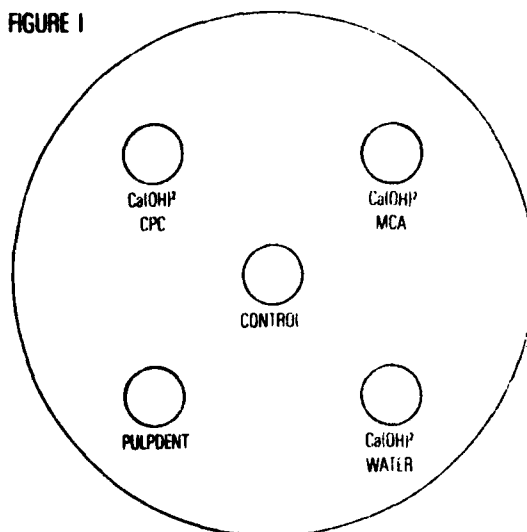
TABLE I

Days	Agent	Diameter of the Zones in mm					Average	SD	Paired t-Test
		1	2	3	4	5			
2	MCA	43.3	38.3	42.8	40.7	35.3	40.08	3.32	<.001
2	CPC	36.8	31.1	28.9	30.2	25.8	30.56	4.06	
Paired Difference		6.5	7.2	13.9	10.5	9.5	9.52		
4	MCA	39.5	36.4	40.4	38.7	28.9	36.78	4.65	<.01
4	CPC	34.6	30.9	27.6	29.1	21.5	28.74	4.82	
Paired Difference		4.9	5.5	12.8	9.6	7.4	8.04		
6	MCA	37.2	34.5	37.6	34.6	24.6	33.70	5.28	<.01
6	CPC	33.8	29.8	26.9	27.5	18.7	27.34	5.84	
Paired Difference		3.4	4.7	10.7	7.1	5.9	6.36		
8	MCA	35.1	32.4	33.9	32.7	22.4	31.30	5.09	<.001
8	CPC	33.2	29.2	26.5	26.9	18.1	26.78	5.53	
Paired Difference		1.9	3.2	7.4	5.8	4.3	4.52		

MCA = Metacresylacetate

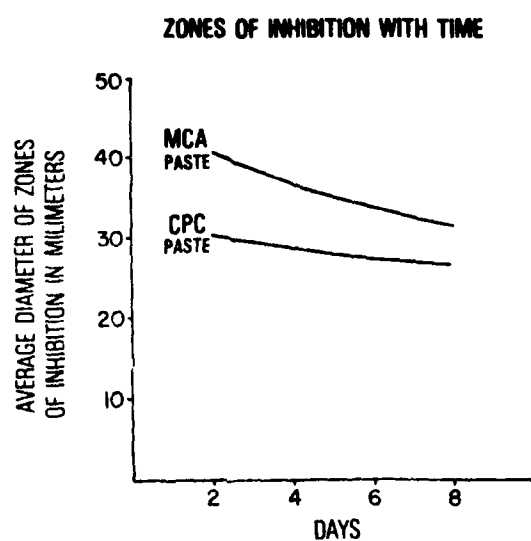
CPC = Camphorated Parachlorophenol

FIGURE I



THE PLACEMENT PATTERN OF PENNY CYLINDERS  
ON THE 15 x 150 mm BLOOD AGAR PLATES





**FIGURE II**  
**GRAPH OF THE AVERAGE DIAMETER OF ZONES OF INHIBITION**  
**AT EACH TIME INTERVAL FOR MCA AND CPC**

MCA - METACRESYLACETATE  
CPC - CAMPHORATED PARACHOLOPHENOL